Biophys J BioFAST, published on December 30, 2004 as doi:10.1529/biophysj.104.057588

Optimizing Transport of Metabolites through Large Channels: Molecular Sieves with and without Binding

Alexander M. Berezhkovskii*# and Sergey M. Bezrukov†

* Mathematical and Statistical Computing Laboratory, Division of Bioscience, Center for Information Technology, National Institutes of Health, Bethesda, MD 20892; † Laboratory of Physical and Structural Biology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892

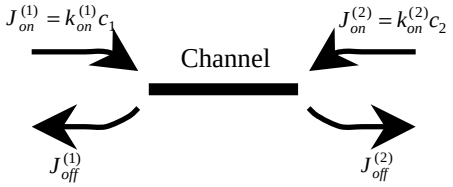
ABSTRACT Using a diffusion model of molecules moving through a pore, we rationalize why biological channels have an affinity for the molecules they have evolved to translocate.

Received for publication "Staff will complete" and in final form "Staff will complete"

Address reprint requests and inquiries to Sergey M. Bezrukov, Tel.: 301-402-4701; FAX: 301-496-2172; E-mail: bezrukov@helix.nih.gov

Membrane channels with large aqueous pores are traditionally regarded as "molecular sieves" which discriminate between different molecules based on their size. This simplified view, however, contradicts emerging experimental evidence that permeation through these structures involves intimate molecular interactions. Metabolite-specific channels exhibit affinity to their metabolites; permeating molecules do not just slip through the pore, but feel strong attraction to the pore-lining residues. The now classical example is bacterial porin LamB (1,2) where the existence of an extended binding zone for oligosaccharides is firmly established. More recent examples include ATP interactions with VDAC (3) and penicillin antibiotic interactions with the general bacterial porin OmpF (4). In this Letter we use a diffusion model for motion of the molecules in the channel (5-7) to rationalize these observations. Contrary to a standard binding-site model, the diffusion model predicts the existence of optimal attraction that maximizes the flux through the channel.

Both the standard two-barrier-one-binding-site model (e.g., (8)) and the diffusion model (5-7) can be represented by the following kinetic scheme



where the incoming fluxes are products of the solute concentrations c_i and the corresponding rate constants $k_{on}^{(i)}$, i = 1,2. The main difference between the two models is how they describe the particle dynamics in the channel. The binding-site model assumes a single-exponential distribution of the molecule lifetime in the channel with the average lifetime τ determined by the rate constants $k_{off}^{(1)}$ and $k_{off}^{(2)}$, $\tau = 1/(k_{off}^{(1)} + k_{off}^{(2)})$. For a symmetric channel $k_{on}^{(1)} = k_{on}^{(2)} = k_{on}$ and $k_{off}^{(1)} = k_{off}^{(2)} = 1/2\tau$. When such a channel can be occupied by only one molecule the flux from the left to the right is

$$J = \frac{k_{on}(c_1 - c_2)}{2[1 + k_{on}(c_1 + c_2)\tau]} \qquad (1)$$

This relation shows that in the binding-site model any increase in the binding strength and, hence, in the molecule lifetime in the channel, decreases the flux. *Why then should channels exhibit affinity to the molecules they have evolved to translocate?*

The diffusion model provides an answer. It shows that there is an optimal well depth which leads to a compromise between sufficiently high translocation probability and not too long blockage of the channel.

The model assumes diffusive motion of molecules inside a cylindrical channel and characterizes their interaction with the channel in terms of the potential of mean force $U(x)$ and the position-dependent diffusion coefficient $D(x)$, where x is a coordinate along the channel axis. Propagation of the molecule in the channel is described by the Green's function $G(x,t;x_0)$ which is

the probability density of finding the molecule at point x at time t on condition that it was at x_0 at t = 0 and it has not escaped from the channel during time t. The Green's function satisfies the Smoluchowski equation

$$\frac{\partial G}{\partial t} = \frac{\partial}{\partial x}\left\{ D(x)\exp\left(-\frac{U(x)}{k_B T}\right)\frac{\partial}{\partial x}\left[\exp\left(\frac{U(x)}{k_B T}\right)G\right]\right\}$$

with the initial condition $G(x,0;x_0) = \delta(x - x_0)$ and radiation boundary conditions (5) at the channel ends. Here k_B and T have their usual meanings.

Assuming that a channel occupied by one molecule is blocked for other molecules, the flux can be written as

$$J = \left(k_{on}^{(1)} c_1 P_{tr}^{(1)} - k_{on}^{(2)} c_2 P_{tr}^{(2)}\right) P_{emp} \qquad (2)$$

where $P_{tr}^{(1)}$ and $P_{tr}^{(2)}$ are the translocation probabilities for molecules entering the channel from the left and right, respectively, and P_{emp} is the probability of finding the channel empty. This probability can be expressed in terms of the average lifetimes of the channel in its empty and occupied states, $P_{emp} = \tau_{emp}/(\tau_{emp} + \tau)$, where $\tau_{emp} = (k_{on}^{(1)} c_1 + k_{on}^{(2)} c_2)^{-1}$ and $\tau = (k_{on}^{(1)} c_1 \tau_1 + k_{on}^{(2)} c_2 \tau_2)\tau_{emp}$. Here τ_1 and τ_2 are the average lifetimes of the molecules in the channel on condition that the molecules enter from the left and right. The translocation probabilities and average lifetimes derived eairlier (6,7) allow one to find the flux for arbitrary $U(x)$ and $D(x)$.

For a symmetric channel with $P_{tr}^{(1)} = P_{tr}^{(2)} = P_{tr}$, $k_{on}^{(1)} = k_{on}^{(2)} = k_{on}$, and $\tau_1 = \tau_2 = \tau$ Eq.2 takes the form

$$J = \frac{k_{on} P_{tr}(c_1 - c_2)}{1 + k_{on}\tau(c_1 + c_2)} \qquad (3)$$

which reduces to Eq.1 if one takes P_{tr} = 1/2 as it should be for a symmetric channel in the framework of the binding-site model.

As has been shown (6), P_{tr} approaches its upper limit of 1/2 when a deep potential well occupies the entire channel. With this in mind, consider a square-well potential of depth U that occupies the entire cylindrical channel of length L and radius R. Additionally, assume that the diffusion coefficient of the molecule in the channel $D(x)$ = const = D_{ch} which can be much smaller than the diffusion coefficient of the molecule in the bulk D_b. In this case general expressions for the translocation probability and average lifetime (6,7) lead to

$$P_{tr} = \frac{1}{2 + \frac{4D_b L}{\pi D_{ch} R}\exp\left(-\frac{U}{k_B T}\right)}, \quad \tau = \frac{\pi R L}{8D_b}\exp\left(\frac{U}{k_B T}\right) \qquad (4)$$

Assuming the diffusion-controlled access of permeating molecules, $k_{on} = 4D_b R$ (9), we arrive at the following expression for the flux in Eq.3

$$J = \frac{2D_b R(c_1 - c_2)}{\left[1 + \frac{\pi R^2 L(c_1 + c_2)}{2}\exp\left(\frac{U}{k_B T}\right)\right]\left[1 + \frac{2D_b L}{\pi D_{ch} R}\exp\left(-\frac{U}{k_B T}\right)\right]} \qquad (5)$$

This expression is one of the main results of this Letter. It shows that the flux depends not only on the geometric parameters of the "molecular sieve" (channel radius R and length L), but also on the strength of the molecule-channel attraction (U) and on the molecule diffusion coefficients (D_b and D_{ch}).

It is important that the flux is a non-monotonic function of the well depth. The depth that maximizes the flux provides a compromise between sufficiently high translocation probability and not too long blockage of the channel. The optimal depth is given by

$$U_{opt} = \frac{k_B T}{2}\ln\left[\frac{4D_b}{\pi^2 D_{ch} R^3 (c_1 + c_2)}\right]. \qquad (6)$$

The optimal well depth depends on the bulk concentration of the translocating molecules because the blockage time should be compared with the inverse frequency of attempts to enter the channel.

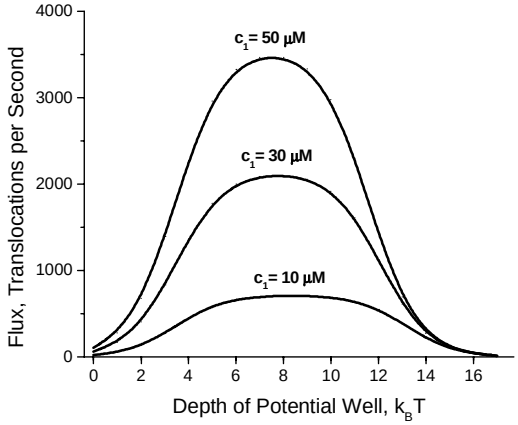


Figure 1. Non-monotonic behavior of the flux given by Eq.5 as a function of potential well depth at three different concentrations of translocating molecules and c_2 = 0.

The non-monotonic behavior of the flux is illustrated by **Figure 1**. The parameters are: (i) L = 5 nm, which is close to the thickness of a lipid bilayer; (ii) R = 0.2 nm, based on the fact that metabolite molecules often demonstrate a tight fit to the channel radius by blocking the small-ion currents almost completely (4,10); because the model describes molecules as point particles, the

parameter R used in Eq.5 is the difference between the radii of the channel and the molecule; (iii) $D_b = 2D_{ch} = 3\text{x}10^{-10}$ m^2/s, following the idea that a molecule in the channel moves somewhat slower than in bulk and using the value of the bulk diffusion coefficient typical for metabolite molecules (e.g., (3)). Figure 1 demonstrates that the optimal well depth depends on the metabolite concentration: the optimum for 50 μM is about one k_BT smaller than the optimum for 10 μM. Importantly, the predicted rates are of the same order of magnitude as those obtained experimentally (3,10).

Substituting U_{opt} given in Eq.3 into Eq.5 we arrive at

$$J_{opt} = \frac{2D_b R(c_1 - c_2)}{\left(1 + L\sqrt{\frac{D_b R(c_1 + c_2)}{D_{ch}}}\right)^2} \qquad (7)$$

To discuss the dependence of J_{opt} and U_{opt} on the concentration of the translocating molecules, we assume that c_2 = 0. At small concentrations the optimal flux increases linearly with c_1. At higher concentrations it saturates, approaching the upper limit $2D_{ch}/L^2$, which is independent of the channel radius. Note, that this result has been obtained assuming that the channel can only be occupied by a single molecule. Concentration at which J_{opt} is equal to 1/2 of its maximum value is $c_1^* = (3+2\sqrt{2})D_{ch}/(D_b R L^2)$. One can check that at this concentration the ratio τ/τ_{emp} is equal to $(1+\sqrt{2})$, i.e., the channel is empty for approximately 30% of the time. Using Eq.6 one can find that at $c_1 = c_1^*$

$$U_{opt}^* = k_B T \ln\left(\frac{2D_b L}{(1+\sqrt{2})\pi D_{ch} R}\right) \qquad (8)$$

Assuming that the diffusion coefficients in the bulk and in the channel do not differ by more than an order of magnitude, i.e., $1 < D_b/D_{ch} < 10$, and taking values of the ratio L/R between 5 and 20, we estimate that the optimal well depth falls in the range of several k_BT 's. This depth provides a compromise between the interaction-induced increase in the translocation probability and decrease in the rate of escape from the channel (Eq.4).

Finally, we note that this Letter addresses only one aspect of the constructive role of attractive interaction between the channel and the translocating molecules since the potential acts on the molecule only inside the channel pore. Generally, attraction between the channel and the molecules may also increase the incoming fluxes (11). However, this aspect of the problem is beyond the scope of our analysis.

Molecular mechanisms by which membrane channels are tuned by evolution to optimize transport of specific solutes are still far from being understood. This is especially true for large, metabolite-specific channels. Though our model is highly idealized as it assumes a uniform pore and allows only single occupancy, we hope that this study will help to clarify the role of attractive interactions between the channel and permeating metabolites.

AKNOWLEDGEMENTS

We thank Adrian Parsegian and Attila Szabo for fruitful discussions.

REFERENCES

(1) Dutzler, R., Y.-F. Wang, P.J. Rizkallah, J.P. Rosenbusch, and T. Schirmer. 1995. Crystal structures of various maltooliosaccharides bound to maltoporin reveal a specific sugar translocation pathway. *Structure* 4:127-134.

(2) Nikaido, H. 2003. Molecular basis of bacterial outer membrane permeability revisited. *Microbiol. Mol. Biol. Rev.* 67:593-656.

(3) Rostovtseva T. K., and S. M. Bezrukov. 1998. ATP transport through a single mitochondrial channel, VDAC, studied by current fluctuation analysis. *Biophys. J.* 74:2365–2373.

(4) Nestorovich, E. M., C. Danelon, M. Winterhalter, S. M. Bezrukov. 2002. Designed to penetrate: Time-resolved interaction of single antibiotic molecules with bacterial pores. *Proc. Natl. Acad. Sci. U.S.A.* 99:9789–9794.

(5) Bezrukov, S. M., Berezhkovskii, A. M., Pustovoit, M. A., and A. Szabo. 2000. Particle number fluctuations in a membrane channel. *J. Chem. Phys.* 113:8206-8211.

(6) Berezhkovskii, A. M., Pustovoit, M. A., and Bezrukov, S. M. 2003. Channel-facilitated membrane transport: Average lifetimes in the channel. *J. Chem. Phys.* 119:3943-3951.

(7) Berezhkovskii, A. M., Pustovoit, M. A., and Bezrukov, S. M. 2002. Channel-facilitated membrane transport: Transit probability and interaction with the channel. *J. Chem. Phys.* 116:9952-9956.

(8) Schwarz, G., C. Danelon, and M. Winterhalter. 2003. On translocation through a membrane channel via an internal binding site: Kinetics and voltage dependence. *Biophys. J.* 84:2990-2998.

(9) Hill, T.L. 1975. Effect of rotation on the diffusion-controlled rate of ligand-protein association. *Proc. Natl. Acad. Sci. U.S.A.* 72:4918-4922.

(10) Kullman, L., M. Winterhalter, and S. M. Bezrukov. 2002. Transport of maltodextrins through Maltoporin: A single-channel study. *Biophys. J.* 82:803-812.

(11) Delcour, A. H. 2003. Solute uptake through general porins. *Front. Biosci*. 8:d1055-1071.

\# Permanent address: *Karpov Institute of Physical Chemistry, Vorontsovo Pole 10, Moscow, Russia 103064*